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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Eaton, et al.
Appl. No.	:	10/063,557
Filed	:	May 2, 2002
For	:	SECRETED AND TRANSMEMBRANE POLYPEPTIDES AND NUCLEIC ACIDS ENCODING THE SAME
Examiner	:	David J. Blanchard
Group Art Unit	:	1642

DECLARATION OF J. CHRISTOPHER GRIMALDI, UNDER 37 CFR §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, J. Christopher Grimaldi, declare and state as follows:

1. I am a Senior Research Associate in the Molecular Biology Department of Genentech, Inc., South San Francisco, CA 94080.

2. My scientific Curriculum Vitae, including my list of publications, is attached to and forms part of this Declaration (Exhibit A).

3. I joined Genentech in January of 1999. From 1999 to 2003, I directed the Cloning Laboratory in the Molecular Biology Department. During this time I directed or performed numerous molecular biology techniques including semi-quantitative Polymerase Chain Reaction (PCR) analyses. I am currently involved, among other projects, in the isolation of genes coding for membrane associated proteins which can be used as targets for antibody therapeutics against cancer. In connection with the above-identified patent application, I personally performed or directed the semi-quantitative PCR gene expression analyses in the assay entitled "Tumor Versus Normal Differential Tissue Expression Distribution," which is described in EXAMPLE 18 in the specification. These studies were used to identify differences in gene expression between tumor tissue and their normal counterparts.

4. EXAMPLE 18 reports the results of the PCR analyses conducted as part of the investigating of several newly discovered DNA sequences. This process included developing

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primers and analyzing expression of the DNA sequences of interest in normal and tumor tissues. The analyses were designed to determine whether a difference exists between gene expression in normal tissues as compared to tumor in the same tissue type.

5. The DNA libraries used in the gene expression studies were made from pooled samples of normal and of tumor tissues. Data from pooled samples is more likely to be accurate than data obtained from a sample from a single individual. That is, the detection of variations in gene expression is likely to represent a more generally relevant condition when pooled samples from normal tissues are compared with pooled samples from tumors in the same tissue type.

6. In differential gene expression studies, one looks for genes whose expression levels differ significantly under different conditions, for example, in normal versus diseased tissue. Thus, I conducted a semi-quantitative analysis of the expression of the DNA sequences of interest in normal versus tumor tissues. Expression levels were graded according to a scale of +, -, and +/- to indicate the amount of the specific signal detected. Using the widely accepted technique of PCR, it was determined whether the polynucleotides tested were more highly expressed, less expressed, or whether expression remained the same in tumor tissue as compared to its normal counterpart. Because this technique relies on the visual detection of ethidium bromide staining of PCR products on agarose gels, it is reasonable to assume that any detectable differences seen between two samples will represent at least a two fold difference in cDNA.

7. The results of the gene expression studies indicate that the genes of interest can be used to differentiate tumor from normal. The precise levels of gene expression are irrelevant; what matters is that there is a relative difference in expression between normal tissue and tumor tissue. The precise type of tumor is also irrelevant; again, the assay was designed to indicate whether a difference exists between normal tissue and tumor tissue of the same type. If a difference is detected, this indicates that the gene and its corresponding polypeptide and antibodies against the polypeptide are useful for diagnostic purposes, to screen samples to differentiate between normal and tumor. Additional studies can then be conducted if further information is desired.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

By: _____ Date: 8/10/2004
J. Christopher Grimaldi

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EXHIBIT A

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J. Christopher Grimaldi

1434-36th Ave.
San Francisco, CA 94122
(415) 681-1639 (Home)

EDUCATION University of California, Berkeley
Bachelor of Arts in Molecular Biology, 1984

EMPLOYMENT EXPERIENCE

SRA Genentech Inc., South San Francisco; 1/99 to present

Previously, was responsible to direct and manage the Cloning Lab. Currently focused on isolating cancer specific genes for the Tumor Antigen (TAP), and Secreted Tumor Protein (STOP) projects for the Oncology Department as well as Immunologically relevant genes for the Immunology Department. Directed a lab of 6 scientists focused on a company-wide team effort to identify and isolate secreted proteins for potential therapeutic use (SPDI). For the SPDI project my duties were, among other things, the critically important coordination of the cloning of thousands of putative genes, by developing a smooth process of communication between the Bioinformatics, Cloning, Sequencing, and Legal teams. Collaborated with several groups to discover novel genes through the Curagen project, a unique differential display methodology. Interacted extensively with the Legal team providing essential data needed for filing patents on novel genes discovered through the SPDI, TAP and Curagen projects. My group has developed, implemented and patented high throughput cloning methodologies that have proven to be essential for the isolation of hundreds of novel genes for the SPDI, TAP and Curagen projects as well as dozens of other smaller projects.

Scientist DNAX Research Institute, Palo Alto; 9/91 to 1/99

Involved in multiple projects aimed at understanding novel genes discovered through bioinformatics studies and functional assays. Developed and patented a method for the specific depletion of eosinophils in vivo using monoclonal antibodies. Developed and implemented essential technical methodologies and provided strategic direction in the areas of expression, cloning, protein purification, general molecular biology, and monoclonal antibody production. Trained and supervised numerous technical staff.

Facilities
Manager Corixa, Redwood City; 5/89 - 7/91.

Directed plant-related activities, which included expansion planning, maintenance, safety, purchasing, inventory control, shipping and receiving, and laboratory management. Designed and implemented the safety program. Also served as liaison to regulatory agencies at the local, state and federal level. Was in charge of property leases, leasehold improvements, etc. Negotiated vendor contracts and directed the purchasing department. Trained and supervised personnel to carry out the above-mentioned duties.

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SRA University of California, San Francisco
Cancer Research Institute; 2/87-4/89.

Was responsible for numerous cloning projects including: studies of somatic hypermutation, studies of AIDS-associated lymphomas, and cloning of t(5;14), t(11;14), and t(8;14) translocations. Focused on the activation of hemopoietic growth factors involved in the t(5;14) translocation in leukemia patients..

Research
Technician Berlex Biosciences, South San Francisco; 7/85-2/87.

Worked on a subunit porcine vaccine directed against Mycoplasma hyopneumoniae. Was responsible for generating genomic libraries, screening with degenerate oligonucleotides, and characterizing and expressing clones in E. coli. Also constructed a general purpose expression vector for use by other scientific teams.

PUBLICATIONS

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MANUSCRIPTS IN PREPARATION

1. Sriram Balasubramanian, J. Christopher Grimaldi, J. Fernando Bazan, Gerard Zurawski and Maureen Howard. "Structural and functional characterization of CD38: Identification of active site residues"

PATENTS

1. "Methods for Eosinophil Depletion with Antibody to CCR3 Receptor" (US 6,207,155 B1).

2. "Amplification Based Cloning Method." (US 6,607,899)

3. Ashkenazi et al., "Secreted and Transmembrane Polypeptides and Nucleic Acids Encoding the Same." (this patent covers several hundred genes)

4. "IL-17 Homologous Polypeptides and Therapeutic Uses Thereof"

5. "Method of Diagnosing and Treating Cartilaginous Disorders."

MEMBERSHIPS AND ACTIVITIES

Editor	Frontiers in Bioscience
Member	DNAX Safety Committee 1991-1999 Biological Safety Affairs Forum (BSAF) 1990-1991 Environmental Law Foundation (ELF) 1990-1991

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BNF-1, a novel gene encoding a putative extracellular matrix protein, is overexpressed in tumor tissues

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Abstract

In an effort to identify novel genes relevant to tumor angiogenesis, we compared the genes expressed in a matched pair composed of vascularized breast tumor and its adjacent normal tissue obtained from the same cancer patient. Using differential display, we identified a cDNA fragment that was reproducibly upregulated in vascularized breast tumor. Up-regulation of this gene fragment in vascularized breast tumor was further verified by semi-quantitative PCR on the same RNA pair using gene-specific primers. The cDNA encoding the full-length ORF of that gene was then cloned by both 3′ and 5′ RACE. Sequence analysis showed that this gene encodes an ORF of 1353 bp having a hydrophobic N-terminal signal sequence and a cleavage site. We named this novel gene BNF-1 (breast tumor novel factor 1). The mature protein of this gene contains cysteine-rich repeats that are a specific feature of several extracellular matrix proteins including thrombospondin-1, thrombospondin-2, pro-collagen type 1, and von Willebrand Factor 1. PCR analysis of BNF-1 expression in a variety of human adult normal tissues revealed that BNF-1 is expressed predominantly in liver, heart, prostate, testis, and ovary. To further study the expression pattern of this novel gene in tumor tissues, we extended our analysis to additional matched pairs of tumor tissues obtained from breast, lung, and colon cancer patients. We show here that BNF-1 is over-expressed not only in breast tumors but also in lung and colon tumors.
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Keywords: Breast cancer; Lung cancer; Colon cancer; Angiogenesis; Cysteine-rich repeats

1. Introduction

The most frequent cause of death in cancer patients results from the metastasis of tumor cells. Many studies have demonstrated that the progression of a tumor and its ability to metastasize are closely associated with the process of tumor angiogenesis (Folkman, 1995). The process of tumor angiogenesis consists of several sequential and interdependent steps that include the degradation of surrounding basement membrane as well as endothelial cell proliferation and migration. Although much has been learned about tumor angiogenesis in the past several years, the molecular events or cascades underlying tumor angiogenesis still remain unclear (Klagsburn and Moses, 1999).

Several studies have suggested that tumor angiogenesis, similar to tumor progression, generally involves altered gene expression in tumor tissues (Black et al., 2001; Benezra, 2001). To elucidate the molecular mechanisms underlying tumor angiogenesis, it is crucial to identify the molecules involved in the angiogenic process and to examine the relationships between them.

A pair of matched vascularized breast tumor and its adjacent normal tissue was obtained from Cooperative Cancer Tissue Registry (National Cancer Institute; Washington DC, USA). mRNAs purified from these tumors were used to construct 1st strand cDNA. Differential displays were then used to identify genes differentially expressed in vascularized breast tumor.

In this study, we report the identification and cDNA cloning of a novel gene, termed BNF-1, that encodes a

Abbreviations: aa, amino acid(s); bp, base pair(s); cDNA, complementary DNA; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); mRNA, messenger RNA; MW, molecular weight; NCI, National Cancer Institute (USA); ORF, open reading frame; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA end(s).

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putative extracellular matrix protein. Sequence analysis revealed that BNF-1 contains a repeated cysteine-rich motif, a special feature of several extracellular matrix proteins, such as thrombospondin-1 (Amagasaki et al., 2001), thrombospondin-2 (Laherty et al., 1992), pro-collagen type 1 (Myers et al., 1981), and Von Willebrand Factor-1 (Sadler et al., 1985). Using semi-quantitative PCR, we showed that BNF-1 is expressed predominantly in adult normal tissues of heart, liver, prostate, testis, and ovary. In addition, the up-regulation of BNF-1 was detected not only in breast tumor tissues, but also in lung and colon tumor tissues.

2. Materials and method

2.1. Breast tumor tissues and RNA purification

A matched pair of breast tumor and its adjacent normal tissue was obtained from Cooperative Cancer Tissue Registry. RNA purification was conducted following standard protocols (Sambrook et al., 1989). In addition, several pairs of RNAs or cDNA obtained from matched breast, lung, and colon tumor/normal tissues were purchased from both Ambion Inc. (Austin, TX, USA), and Clontech (Palo Alto, CA, USA).

2.2. Differential display

In this study, differential display was performed as previously described (Wu and Moses, 2001). Briefly, mRNA was purified from both breast tumor and its adjacent normal tissue which were obtained from Cooperative Cancer Tissue Registry. The 1st strand cDNA was constructed using oligonucleotides 15 mers, TTTTTTTTTTTTTTT. PCR amplifications were then carried out by using arbitrary oligonucleotides 10 mers. The PCR band that was determined to be amplified from differentially expressed cDNA was excised from the gel. cDNA was then extracted from the excised piece of gel and used as template for PCR using the same arbitrary primer. The reamplified DNA was subcloned into pCR II (Invitrogen, Carlsbad, CA, USA) and sequenced using an UBI 373 sequencer (Applied Biosystem, San Jose, CA, USA).

2.3. Molecular cloning of a cDNA encoding the full-length ORF of BNF-1

The BNF-1 cDNA fragment obtained from differential display was subcloned into the pCRII vector using the TA cloning system (Invitrogen). The differential expression was confirmed by semi-quantitative PCR according to the protocol described previously (Wu and Moses, 1998, 2001) using a pair of gene specific primers (forward 5′-GTCCTGAAGGAGAAACATAAGAAAG-3′ and backward 5′-CATCCTCACAGGTGCATAGGATGCAG-3′) from the same RNA sample used for differential display. The full-length cDNA of BNF-1 was cloned by both 3′ and 5′ RACE as reported earlier (Wu and Moses, 1996, 1998). 3′ RACE reactions were made with sense primers 5′-GAGAAACATAAGAAAGCCTGTGTGCATG-3′ and nested sense primers 5′-CACAGTGAGATCAGTTCTAC-CAGGTGTC-3′. 5′ RACE reactions were made with antisense primer 5′-CTTGGGACACCTGGTAGAACT-GATCAC-3′ and nested antisense primer 5′-CAAATCTTGCAGCACTTCCCAGCCAG-3′. pCR II (Invitrogen) was used for cloning and sequencing reactions. Both the forward and the reverse strands of the cDNA were sequenced to determine the complete cDNA sequence.

2.4. DNA sequencing and analysis

DNA sequencing was conducted using a fluorescence-labeled automatic sequencing apparatus, Model 373 DNA sequencer. Sequence analyses of the nucleotide and protein sequences were performed using the Wisconsin Genetic Control Group program package (Genetics Control Group, Madison, WI, USA).

2.5. Expression of BNF-1 in human tissues

Tissue-specific expression of BNF-1 was characterized by semi-quantitative PCR according to the protocol described previously (Wu and Moses, 1998, 2001). PCR amplification of first-strand cDNA was performed with gene-specific primers (forward 5′-CAGGATCCATGTTC-CAGTGATGCTG-3′ and backward 5′-CTTCAGGAC-GATCTTGACAGTTGTG-3′) on a normalized human Multiple Tissue cDNA panel (Clontech).

2.6. Tumor tissue expression pattern of BNF-1

cDNAs from several tumor tissues were constructed or obtained from Commercial Companies (Ambion and Clontech). cDNAs were normalized to ribosome binding protein-9. Semi-quantitative PCR were conducted according to the protocol described previously (Wu and Moses, 1998, 2001). The primer pair used was the forward primer 5′-CAGGATCCATGTTCCAGTGATGCTG-3′ and the backward primer 5′-CTTCAGGACGATCTTGA-CAGTTGTG-3′.

3. Results

3.1. Identification of BNF-1

As the first step in identifying novel genes whose expression might correlate with the development of tumor angiogenesis, we compared the repertoire of genes expressed by the vascularized breast tumor tissue and its adjacent normal tissue obtained from the same patient. Total

RNAs were purified from frozen tumor and normal tissues, which were treated with DNase I, followed by the construction of the 1st strand cDNA. Using differential display, we conducted hundreds of reactions using distinct primers against these two tissue types. Among these PCR amplifications, using an arbitrary primer DDCA1 (5′-CATGTTCCAG-3′), we identified a cDNA fragment that appeared to be expressed only in vascularized tumor tissue as compared to its adjacent normal tissue (Fig. 1A). The reaction showing a differentially expressed band was repeated twice to ensure reproducibility. This differential display 'band' was purified, reamplified, and cloned into pCR II vector, which was then sequenced. Sequencing analysis of the cDNA fragment revealed it to be 337-bp in length with no similarity to any of the sequences in the published nucleotide database. The specific expression pattern of the cDNA fragment was further confirmed by semi-quantitative RT-PCR using gene-specific primers and the RNAs previously used for differential display (Fig. 1B). To obtain a cDNA encoding the full-length ORF of BNF-1, both 5′ and 3′-RACE were carried out following a protocol used previously (Wu and Moses, 1996, 1998). The longest cDNA fragment obtained by both 5′ and 3′-RACE was then cloned and sequenced.

3.2. Sequence analysis of BNF-1

Sequence analysis of the longest cDNA fragment reveals that it contains a single open reading frame of 1353 bp which encodes a polypeptide of 451 amino acid residues, with the first ATG lying in a favorable context for initiation of transcription and translation (AGGATG) (Fig. 2A). This novel gene is named BNF-1. Hydropathy analysis using

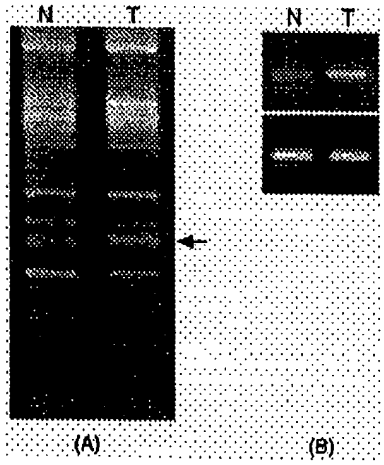


Fig. 1. Identification of a differentially expressed BNF-1 gene fragment in vascularized breast tumor tissue and its adjacent normal tissue. (A) Differential display PCR using a random primer described in material and method. The arrow indicates the BNF-1 gene fragment up-regulated in vascularized breast tumor tissue. (B) Confirmation of differential display-PCR by semi-quantitative PCR using the BNF-1 gene specific primer set. The expression of ribosome protein S9 was analyzed as a normal control.

PSORT II (Nakai and Kanehisa, 1992) and SignalP (Nealsen et al., 1999) showed a single hydrophobic segment, in the amino terminus, comprising the 25 amino acid residues, followed by a signal peptide cleavage site. The presence of a signal peptide and the lack of a possible transmembrane segment suggest that BNF-1 encodes a secreted protein. Mature BNF-1 has a predicted molecular weight of 49,600 Da (MW 49.6 K).

Sequence homology searches of all available databases revealed no significant homology to known genes. However, sequence analysis using Vector NTI Suite (Infomax, Bethesda, MD, USA) revealed that BNF-1 contains three cysteine-rich repeats (Fig. 2A). Each repeat contains nine cysteine residues at conserved positions. (Fig. 2B).

When the cysteine-rich repeats were used to search the BLAST network data base, it was found that the similar cysteine-rich repeats were present in several extracellular matrix proteins, including thrombospondin 1, thrombospondin 2, procollagen type 1 and von Willebrand factor 1 (Fig. 2B). The conservation is restricted primarily to the spacing of cysteine-residues.

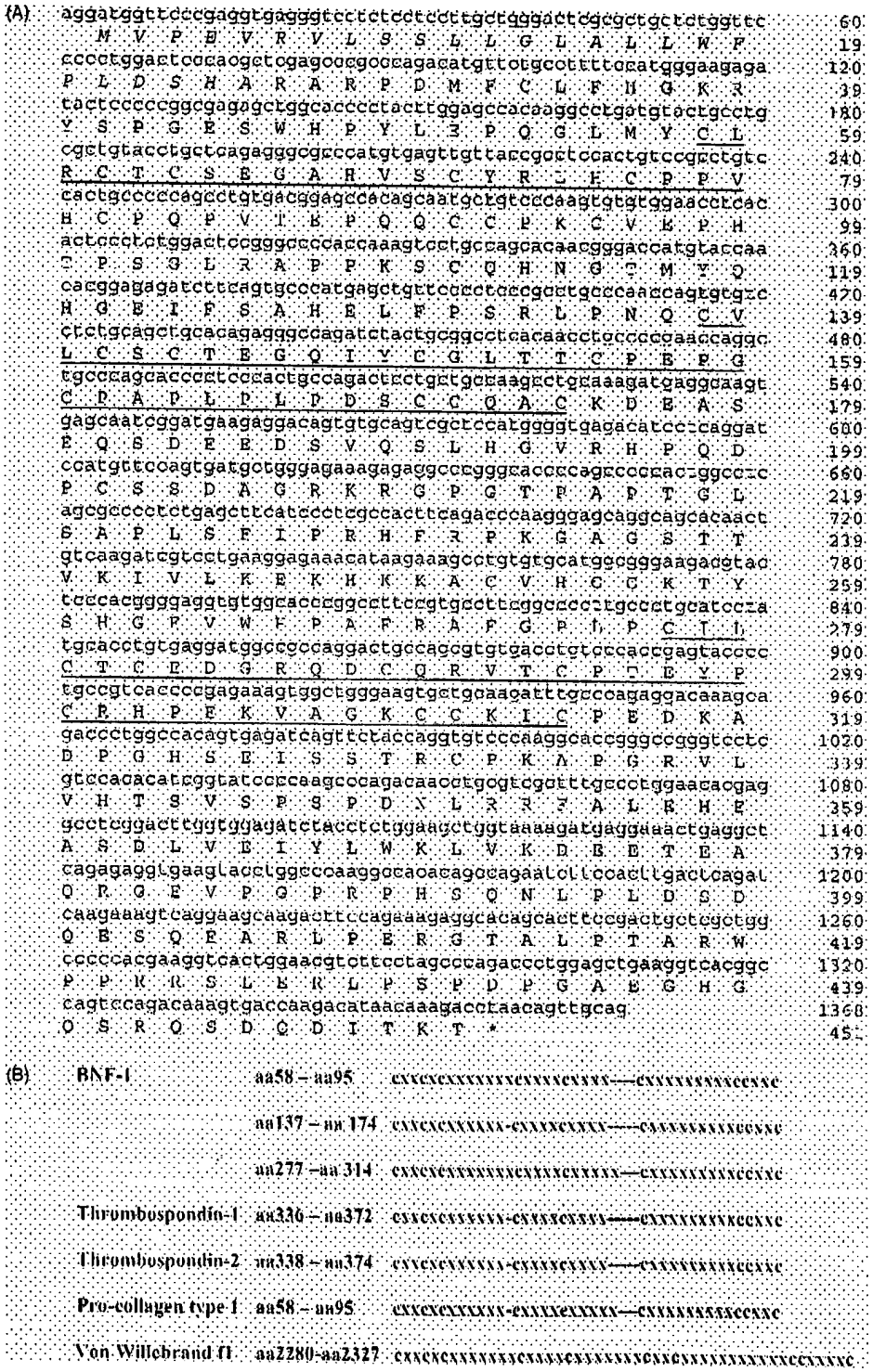
3.3. BNF-1 expression in normal adult tissues

In order to determine the tissue expression pattern of BNF-1, we obtained a cDNA panel that contains normalized cDNA constructed from mRNA purified from 16 distinct human adult normal tissues. Semi-quantitative PCR amplifications were then conducted using a set of gene-specific primers and the cDNA panel as template. The result has shown that BNF-1 has a very specific expression pattern (Fig. 3). It is moderately expressed in heart, liver, prostate, testis, and ovary. Low-levels expression was detected in skeletal muscle, kidney, spleen, small intestine, and colon. However, BNF-1 was not detected in brain, placenta, lung, pancreas, thymus, and peripheral blood leukocytes. This specific pattern of expression suggests that the function of BNF-1 in normal tissues might be tissue-type related.

3.4. Expression pattern of BNF-1 in breast, colon, and lung tumor tissues

We next examined whether BNF-1 was upregulated in other tumor tissues, in addition to the matched pair of breast tumor/normal tissue provided by NCI. We obtained or constructed pairs of cDNA from closely matched breast, colon, and lung tumor/normal tissues, in which each pair of tissues was obtained from the same patient. Semi-quantitative PCR amplifications were then conducted as described earlier. Among the ten pairs of breast tumor/normal samples, BNF-1 was shown to be up regulated about 2- to 3-fold in two tumor tissues as shown in Fig. 4A. Taken together, BNF-1 was detected to be up regulated in approximately 27% (3/11) of breast tumor tissues obtained by us, including the original sample.

BNF-1 was also found to be over-expressed in lung



(B)

BNF-1	aa58 – aa95	CXXCXCXXXXXXXCXXXXCXXXX—CXXXXXXXXXCCXXC
	aa137 – aa174	CXXCXCXXXXXX-CXXXXCXXXX—CXXXXXXXXXCCXXC
	aa277 – aa314	CXXCXCXXXXXXXCXXXXCXXXXX—CXXXXXXXXXCCXXC
Thrombospondin-1	aa336 – aa372	CXXCXCXXXXXX-CXXXXCXXXX—CXXXXXXXXXCCXXC
Thrombospondin-2	aa338 – aa374	CXXCXCXXXXXX-CXXXXCXXXX—CXXXXXXXXXCCXXC
Pro-collagen type I	aa58 – aa95	CXXCXCXXXXXX-CXXXXCXXXX—CXXXXXXXXXCCXXC
Von Willebrand II	aa2280-aa2327	CXXCXCXXXXXXXCXXXXCXXXXXXXCXXCXXXXXXXXXXCCXXXC

Fig. 2. (A) Complete protein coding sequence of human BNF-1. The N-terminal hydrophobic leader sequence is italicized. The cysteine-rich repeats are underlined. GenBank accession number is AY163868. (B) Amino acid alignment of the cysteine-rich repeats of BNF-1 with those in human thrombospondin-1, thrombospondin-2, pro-collagen type 1, and von Willebrand Factor 1.

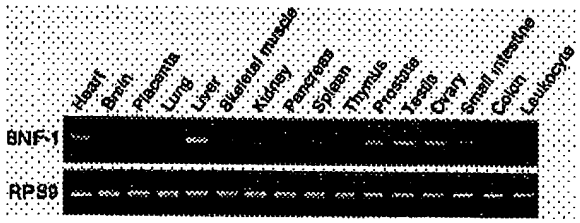


Fig. 3. Expression of BNF-1 mRNA in human normal tissues. Semi-quantitative PCR was performed on human multiple-tissue cDNA panels (Clontech) from the indicated adult tissues. The expression of ribosome protein S9 was analyzed as a normal control using a commercial primer kit (Clontech).

tumors vs. their adjacent normal tissues. These results have shown that approximately 2- to 3-fold up-regulation of BNF-1 expression in about 30% (2/6) of lung tumor tissues obtained (Fig. 4B).

The levels of BNF-1 expression in RNAs isolated from six colon tumors and their matched normal tissues were also assessed by semi-quantitative PCR. The level of BNF-1 was up-regulated approximately 2- to 4-fold in about 16% (1/6) of the tumor tissues examined compared with normal adjacent normal colon tissues (Fig. 4C).

Interestingly, we observed the up-regulation of BNF-1 not only in breast cancer patients, but also in lung and colon cancer patients, which suggests that the over-expression of BNF-1 is independent of specific tumor type. However, the

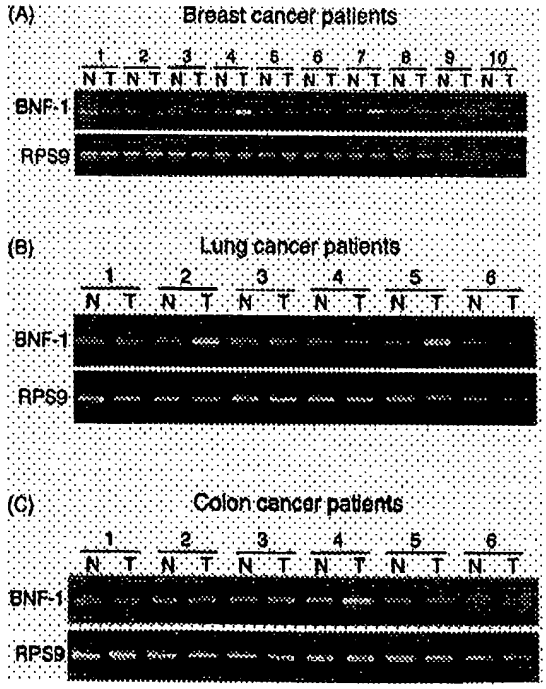


Fig. 4. Expression of BNF-1 mRNA in (A) breast normal/tumor tissues, (B) lung normal/tumor tissues, and (C) colon normal/tumor tissues. Semi-quantitative PCR was performed on cDNA pairs constructed from closely matched normal/tumor tissues. The expression of ribosome protein S9 was analyzed as a normal control using a commercial primer kit (Clontech).

pathological information provided by commercial companies for these tumor RNAs and cDNAs does not include the vascularized state of the tumor tissues. Therefore, the relationship between the up-regulation of BNF-1 in tumor tissues and tumor vascularization is not determined in this study.

4. Discussion

In this study, we have cloned a novel gene, BNF-1, whose cDNA fragment was originally identified as being differentially expressed in matched tumor/normal tissue from a breast cancer patient. Sequence analysis of the full-length ORF of BNF-1 revealed that BNF-1 contains a N-terminus signal peptide followed by a cleavage site. The mature protein of BNF-1 lacks a putative transmembrane domain, which suggests that BNF-1 encodes a secreted protein. In addition, protein sequence analysis revealed that mature BNF-1 contains cysteine-rich repeats, a specific feature of several other extracellular matrix proteins including thrombospondin-1, thrombospondin-2, pro-collagen type 1, and von Willebrand Factor 1. Taken together, these data suggest that BNF-1 may encode a putative extracellular matrix protein. Evidence is provided in this report that BNF-1 has a specific expression pattern in adult normal tissues. Moderate expression of BNF-1 was detected only in heart and liver, as well as some hormone-dependent tissues, such as ovary, testis, and prostate. This specific pattern of expression suggests that the function of BNF-1 in normal tissues might be tissue-type related.

To further confirm the expression pattern of BNF-1 in different types of tumor tissues, we extended our analysis to ten additional breast patients, six additional lung cancer patients, and six additional colon patients. In this study, we chose to use tumor tissues, instead of cancer cell lines, since reports in the literature demonstrate the loss of tumor markers associated with continuous tissue culture of some cancer cell lines (Luyten et al., 1996; Mira-y-Lopez and Ossowski, 1990). However, the use of tumor tissues obtained from breast, lung, and colon cancer patients is not without issue. For example, it is a well-recognized phenomenon that tissue heterogeneity is common among solid tumor tissues (Vescio et al, 1990). Interestingly, we observed the up-regulation of BNF-1 not only in breast cancer patients, but also in lung and colon cancer patients, which suggests that the over-expression of BNF-1 is independent of specific tumor type. In this study, the frequency of upregulation of BNF-1 varied among breast, lung, and colon tumors. It was, despite the limited sample size, consistent with that reported for some oncogenes in solid tumors. For example, it has been reported that oncogene N-MYC was amplified in about 6–7% of small cell lung tumors, and oncogene L-MYC was amplified in about 13% small cell lung tumors (Nau et al., 1985). The clinical information provided for these commercially

available tumor RNAs and cDNA does not include the vascular status of the tumor tissues, therefore the relationship between the up-regulation of BNF-1 in tumor tissue and tumor vascularization remains unclear.

In conclusion, we have isolated a full-length novel human gene, BNF-1, whose cDNA fragment was originally identified as being differentially expressed in matched tumor/normal tissue from a breast cancer patient. Sequence analysis revealed that BNF-1 contains a N-terminus signal peptide. In addition, protein sequence analysis revealed that mature BNF-1 contains cysteine-rich repeats, a specific feature of several other extracellular matrix proteins. In the adult normal tissues, BNF-1 has a specific expression pattern in which it is expressed at relatively moderate levels in heart, liver, prostate, testis, and ovary. It is of interest that BNF-1 was not only up-regulated in breast tumors, but also in lung and colon tumors.

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